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AUTHOR: Bedrosian, I.; et al
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TITLE: Implantation of metastasis. . .
AUTHOR: Jacobi C A; et al
SOURCE: **Surg. Endosc., (1995) 9, 351-2.**

TITLE: Sustained anti-adherence activity. . .
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# TAUROLIDINE, AN ANALOGUE OF THE AMINO ACID TAURINE, SUPPRESSES INTERLEUKIN 1 AND TUMOR NECROSIS FACTOR SYNTHESIS IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Charles A. Dinarello[1]

**Taurolidine (Geistlich Pharm, AG, Wolhusen, Switzerland), a derivative of the amino acid taurine, is commonly used in some parts of the world as an adjunctive therapy for various infections. Its mechanism of action is thought to be related to its antimicrobial properties, including its ability to interfere with some of the biological activities of endotoxin (lipopolysaccharide, LPS). For example, taurolidine has been shown to protect animals against endotoxic shock and death. In this study we examined the ability of taurolidine to block LPS-induced tumor necrosis factor (TNF) and interleukin 1 (IL-1) synthesis in human peripheral blood mononuclear cells (PBMC) from 27 donors. We observed a dose-dependent reduction in the synthesis of these two cytokines when taurolidine was preincubated with LPS before being added to PBMC. This reduction was independent of the molar ratio of taurolidine to LPS but was related to the concentration of taurolidine present in the PBMC cultures. There was a 80 to 90% reduction in total IL-1 and TNF synthesis induced by LPS at concentrations of taurolidine of 40 to 100 μg/mL; the vehicle was without effect. Following a 30-min preincubation with PBMC, taurolidine could be washed from the cells and still suppress cytokine synthesis induced by LPS. Using release of lactic acid dehydrogenase, 100 μg/mL of taurolidine was not toxic for PBMC. Taurolidine also reduced IL-1 and TNF synthesis induced by the *Staphylococcus aureus*–derived toxic shock syndrome toxin-1 as well as that induced by nontoxic heat-killed *Staphylococcus epidermidis* organisms. These results show that taurolidine blocks the production of IL-1 and TNF in human PBMC; furthermore, they suggest that the protective effect of taurolidine may, in part, be due to its ability to reduce IL-1 and TNF synthesis during infection.**


Despite effective antibiotic and supportive therapy, acute sepsis is still associated with significant mortality. Mortality in septic patients is often the result of septic shock in which hypotension, tissue damage, and organ failure occur in rapid succession. Studies in animal models of shock have shown that tumor necrosis factor (TNF), a polypeptide cytokine synthesized in response to infection and injury, can produce a nearly identical syndrome as bacterial-induced shock, with hypotension, acidosis, capillary leak syndrome, tissue necrosis, organ failure, and death when injected into healthy animals.[1] Interleukin 1 (IL-1), also a polypeptide cytokine with many of its biological properties similar to those of TNF,[2] acts synergistically with TNF in producing lethal shock and death in experimental animals.[3] In humans with septic shock, levels of TNF often correlate with the severity of disease.[4] Elevated levels of IL-1 are also found in severe infections.[5]

Using antibodies to TNF[6] or a receptor antagonist to IL-1,[7] hypotension and death in experimental animals can be prevented during endotoxemia or *Escherichia coli* sepsis. In addition, antibodies to the IL-1 receptor type I prevent weight loss and inflammation due to tissue necrosis,[8] and infusions of the IL-1 receptor antagonist reduce inflammation in a model of

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colitis.[9] These studies support the concept that reduction in the synthesis of TNF and IL-1 during sepsis would be beneficial. Agents that suppress the synthesis of these cytokines include corticosteroids[10] and inhibitors of the lipoxygenase pathway of arachidonic acid metabolism[11]; prior treatment of animals with either corticosteroids or inhibitors of lipoxygenase metabolites has been shown to attenuate fever, shock, and death. However, large doses or corticosteroids are associated with unwanted side effects, and this therapy has not been successful in preventing death in humans with bacterial shock. Therefore, agents have been sought which suppress the synthesis of IL-1 and TNF as putative therapies for the prevention or treatment of shock.

Taurolidine (Geitslich Pharm, AG, Wolhusen, Switzerland), an analogue of the amino acid taurine, has been used in humans with a variety of infections as an antimicrobial and antitoxic agent.[12-14] Large amounts have been administered to humans without demonstrable side effects. Taurolin is formed by the condensation of two molecules of the amino acid taurine with three molecules of formaldehyde. In aqueous solutions, taurolidine undergoes hydrolysis liberating free methylol groups ($CH_2OH$) which cross-link proteins resulting in various degrees of inactivation.[15] Following hydrolysis, the amino acid derivatives are then metabolized back to taurine. The cross-linking of bacterial membranes and functional proteins is thought to be the mechanism by which taurolidine prossesses antimicrobial properties.[16]

Studies have also been performed using bacterial endotoxin (lipopolysaccharide, LPS) in animals. Taurolidine is effective in reducing the mortality rate due to LPS in mice.[17] In rabbits administered LPS, fever is reduced by pretreatment with taurolidine. The mechanism by which taurolidine reduces the biological activity of LPS is thought to be due it ability to irreversibly cross-link the protein moieties of LPS.[18]

Humans receive as much as 5 grams of taurolidine intravenously over a 2- to 3-h period without demonstrable side effects.[14] Although taurolidine does not influence the chromogenic Limulus amebocyte lysate (LAL) test,[19] intravenous administration of taurolidine during endotoxemia was associated with a reduction in circulating LPS.[14] In septic humans, data suggest that taurolidine might be effective in the treatment of septic shock not only because of its antimicrobial properties but also because it inactivates bacterial toxins, particularly LPS. Inactivation of the ability of LPS to induce the synthesis of TNF and IL-1 would seem to be a plausible hypothesis to explain its protective effects in animal models of sepsis.[16-18]

In this study, we examined the ability of taurolidine to affect the production of TNF and IL-1 from human peripheral blood mononuclear cells (PBMC) stimulated in vitro by LPS as well as other inducers of cytokine synthesis. The data suggest that taurolidine suppresses the production of these two cytokines by a mechanism which is independent of inactivating bacterial toxins.

## RESULTS

### *Effect of Preincubation With Taurolidine on Lipopolysaccharide-Induced Interleukin 1 and Tumor Necrosis Factor Synthesis*

To test the hypothesis that taurolidine inactivated LPS, we examined whether prior incubation of LPS with taurolidine would affect the ability of LPS to induce IL-1 or TNF in PBMC. LPS was incubated for 60 min at 37°C in either in RPMI or RPMI containing various concentrations of taurolidine.

The initial experiments used a concentration of LPS of 10 ng/mL with LPS:taurolidine weight ratios of 1:1,000 (10 μg/mL of taurolidine); 1:10,000 (100 μg/mL); or 1:100,000 (1 mg/mL). As shown in Fig. 1A, there is a dose-dependent decrease in the amount of TNF produced with increasing concentrations of taurolidine. A 1:10,000 ratio of LPS to taurolidine (100 μg/mL) reduced TNF production to 13% of control. Similar results were observed for IL-1β (Fig. 1B) in these same cultures, although there was a rapid decrease to 100% inhibition at a 1:10,000 weight ratio comparable to 100 μg/mL of taurolidine.

Without changing the ratios of LPS to taurolidine, we repeated these experiments using less LPS (1 ng/mL). Figure 2A shows TNF levels and Fig. 2B shows IL-1β levels. At a 1:10,000 ratio, TNF production is 85% and IL-1β is 80% of the LPS control. These data suggest that the ratio of LPS to taurolidine during the preincubation is not critical but rather the absolute concentration of taurolidine present in the PBMC cultures determines the extent of inhibition of cytokine synthesis.

In another five donors, we examined the effect of taurolidine at 5 and 10 mg/mL on LPS-stimulated (10 ng/mL) cytokine production and observed 100% inhibition of IL-1β production and 90% inhibition of TNF. However, these concentrations of taurolidine may be toxic to PBMC. The vehicle (kollidon; I.G. Farben-BASF, Ludwigshafen, Germany) was tested at concentrations comparable to that present in taurolidine at 100 μg and 1 mg/mL. Figure 3 shows these data. LPS-induced TNF production was significantly inhibited to 7.8 and 4.6% of the LPS control value by taurolidine concentrations of 100 μg and 1 mg/mL, respectively. However, comparable concentrations of the vehicle kollidon had little effect. Therefore, it appears that concentrations of 100 μg or 1 mg/mL of

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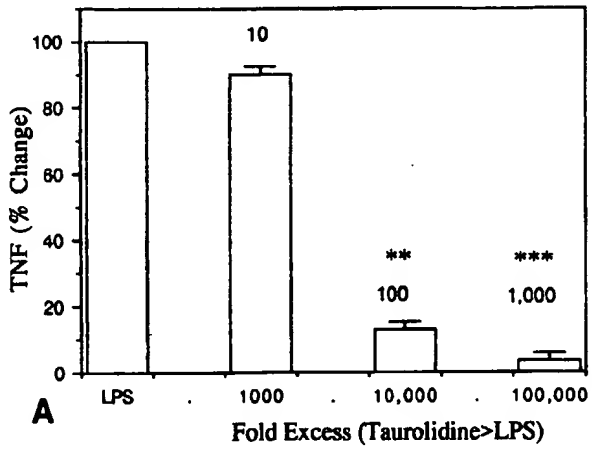


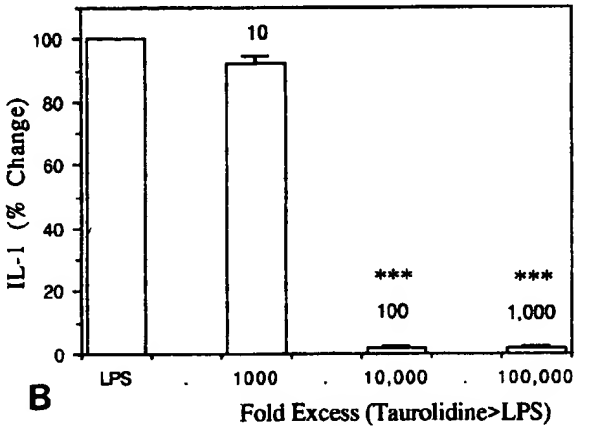




**Figure 1. The effect of taurolidine-LPS mixtures on IL-1 and TNF synthesis in PBMC.**

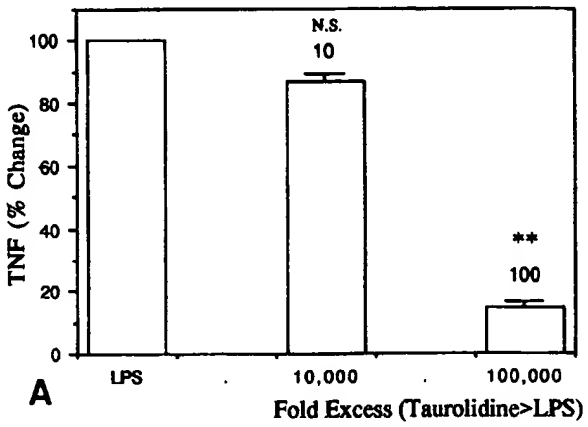
(A) TNF; (B) IL-1. The results are shown as the mean percent change (±SEM) in cytokine synthesis in PBMC from six donors. The amount of cytokine synthesized by 10 ng/mL of LPS without taurolidine was set at 100% for each donor. The numbers above the bars indicate the final concentration of taurolidine per milliliter in each PBMC incubation. $^{**}P < 0.01$; $^{***}P < 0.001$.

taurolidine are suppressing cytokine production which is not due to the vehicle.

Next, we examined the release of lactic acid dehydrogenase as a measure of cytotoxicity. PBMC were incubated with LPS plus increasing concentrations of taurolidine from 10 to 100 μg/mL. As shown in Fig. 4, taurolidine at concentrations of 40 to 100 μg/mL significantly suppressed TNF production, whereas lactic acid dehydrogenase (LDH) released into the PBMC supernate was unchanged. At a concentration of 100 μg/mL of taurolidine, there was an increase from control value of 6 to 10%, but this was not statistically significant. When toxic shock syndrome toxin-1 (TSST-1) was used as the stimulant (see below), LDH release in the presence of 100 μg/mL of taurolidine was 3%. We conclude that taurolidine at 100 μg/mL reduces cytokine production without being toxic to the cells.

### *Effect of Taurolidine on* Staphylococcus epidermidis *and Toxic Shock Syndrome Toxin-1–Induced Cytokine Production*

Heat-killed *Staphylococcus epidermidis* organisms were preincubated with taurolidine at 200 μg/mL for 60 min at 37°C and then added to equal volumes of PBMC. Fig. 5 illustrates the ability of taurolidine to inhibit the TNF induction by this phagocytic stimulus in four donors. In two donors, the reduction was



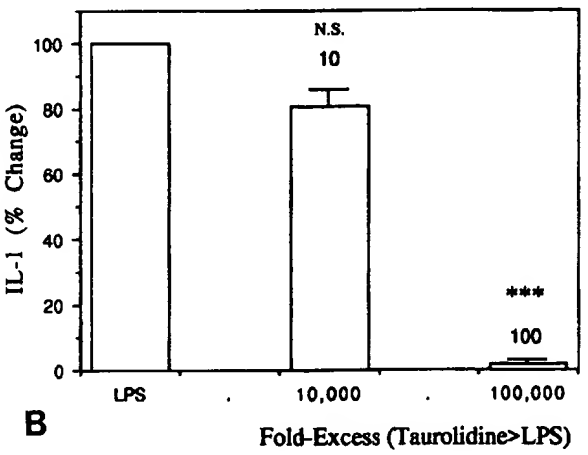




**Figure 2. The effect of taurolidine-LPS mixtures on IL-1 and TNF synthesis in PBMC.**

(A) TNF; (B) IL-1. Data are derived from six donors shown in Fig. 1 except the LPS concentration was 1 ng/mL (final concentration in the PBMC cultures). The numbers above the bars indicate the final concentration of taurolidine in micrograms per milliliter in each PBMC incubation. $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant.

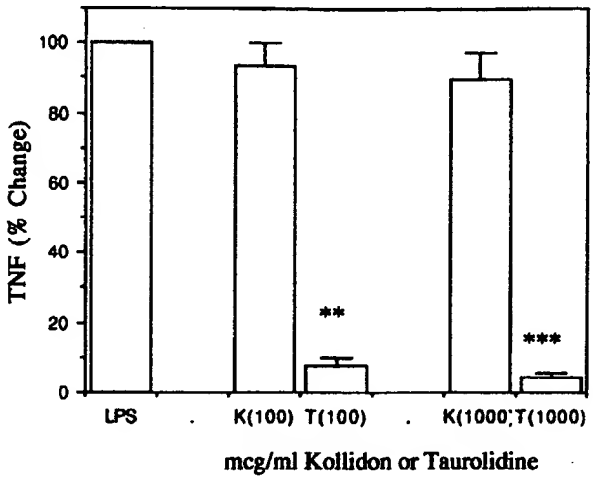



**Figure 3. Comparison of taurolidine and kollidon on LPS-induced TNF production.**

Mixtures of taurolidine (T) or comparable concentrations of kollidon (K) were made with LPS at 20 ng/mL and incubated for 60 min at 37°C. Thereafter, these mixtures were added to equal volumes of PBMC and cultured overnight. The data represent mean percent change (±SEM) in cytokine synthesis in PBMC from four donors. $^{**}P < 0.01$; $^{***}P < 0.001$.

greater than 98% but in the other two donors, the reduction was approximately 50%.

Figure 6A depicts the effect of preincubation of taurolidine with TSST-1 on TNF production. At 10 μg/mL of taurolidine, there is an increase in the amount of TNF followed by inhibition at concentrations of 40, 80, and 100 μg/mL. As shown in Fig. 6B, the increase in cytokine production at 10, 20, and 40 μg/mL is more pronounced. The decrease occurs at taurolidine concentrations of 80 and 100 μg/mL.

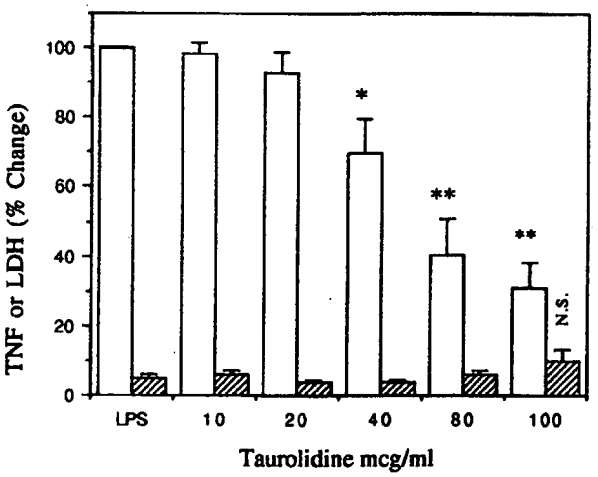



**Figure 4. Comparison of taurolidine-induced suppression of TNF (□) synthesis with release of LDH (▨) in PBMC.**

PBMC from three donors was stimulated with LPS (10 ng/mL)-taurolidine mixtures, and after 24 h of incubation, the TNF and LDH levels were measured. LDH is expressed as the percent of LDH detected in the supernatant medium compared to the total intracellular pool of LDH measured after cell lysis. The levels of TNF in these same samples represent mean percent change (±SEM) in cytokine synthesis in PBMC from these three donors. $^{*}P < 0.05$, $^{**}P < 0.01$, Student's $t$ test. N.S., not significant.

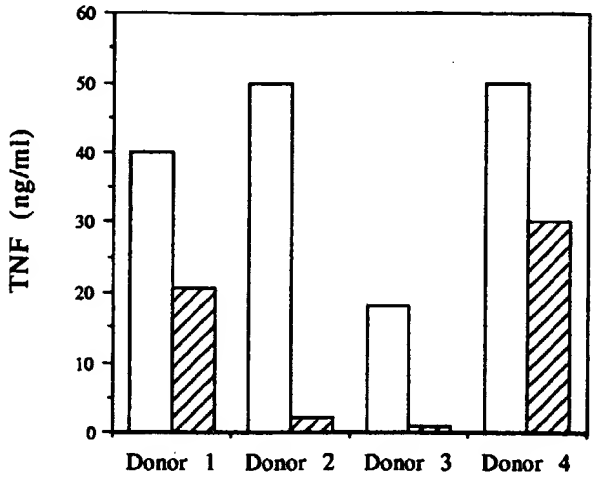



**Figure 5. Effect of taurolidine-*Staphylococcus epidermidis* mixtures on TNF synthesis.**

Taurolidine was mixed with *S. epidermidis* organisms and after 60 min at 37°C, was added to equal volumes of PBMC. After 24 h, the TNF levels were measured. The individual levels in ng/mL of TNF for each of the four donors are shown. □, staph; ▨ staph + T.

### *Effect of Preincubation of Peripheral Blood Mononuclear Cells With Taurolidine Before Stimulation With Lipopolysaccharide*

Because the data shown in Figs. 1 and 2 suggest that the effect of taurolidine was on the cell's ability to produce cytokines rather than an effect of taurolidine preincubation with the stimuli, we incubated PBMC to increasing concentrations of taurolidine (10 to 100 μg/mL) at 37°C for 30 min. The cells were then stimulated with LPS (10 ng/mL) and cytokine determinations made after 24 h. As shown in Fig. 7, the dose-response of taurolidine was shifted towards the left and reduction of over 90% in the production of IL-1 and TNF was observed at taurolidine concentrations of 40, 80, and 100 μg/mL. These data suggest that the effect of taurolidine is to decrease the ability of PBMCs to produce IL-1 and TNF without being toxic.

To determine whether such decreases in PBMC IL-1 and TNF production require the continuous presence of taurolidine, cells were incubated for 30 min with taurolidine (80,000 ng/mL), centrifuged at 300 rpm, and resuspended in an equal amount of RPMI. Except for the control, all cells were then challenged with LPS (1 ng/mL) and cytokine levels were determined after 24 h of incubation. The results shown in Fig. 8 indicate that a 30-min exposure to taurolidine is as effective at suppressing cytokine synthesis as continuous presence of the compound.

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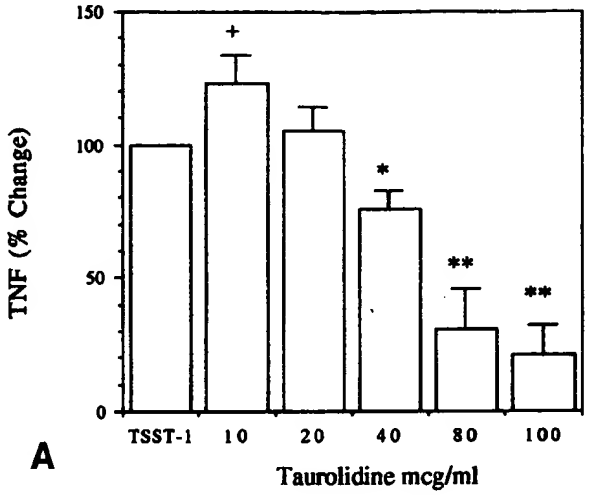


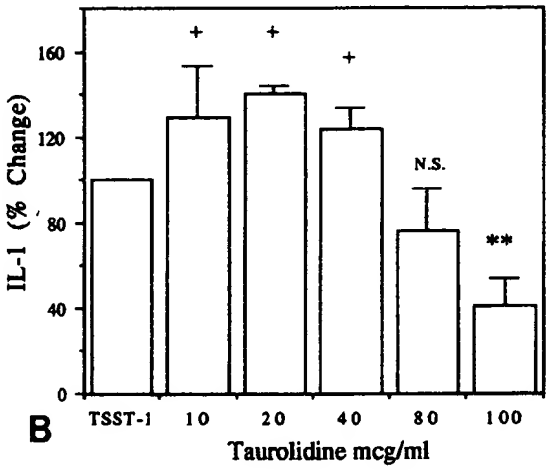


**Figure 6. Effect of mixtures of taurolidine and TSST-1 on IL-1 and TNF synthesis.**

TSST-1 was mixed with increasing concentrations of taurolidine and after 60 min at 37°C was added to equal volumes of PBMC. (A) TNF; (B) IL-1. The data represent the mean percent change (±SEM) in cytokine synthesis in PBMC from four donors. $^{*}P < 0.05$ for cytokine synthesis above the control value; $^{**}P < 0.01$ represents levels of significance of cytokine production below the control value.

## DISCUSSION

Ample evidence indicates that taurolidine possesses antibacterial properties; in addition, because of its antitoxic properties, the agent can also reduce the biological activity of LPS in vivo.[12,16-18] However, present studies show that the compound suppresses the synthesis of IL-1β and TNF-α. The suppression of LPS-induced cytokines was independent of inactivation of LPS and was also observed with non-LPS and nontoxic cytokine-inducing agents. These findings suggest that some of the beneficial effects of taurolidine in bacterial infections may be related to its capacity to suppress the

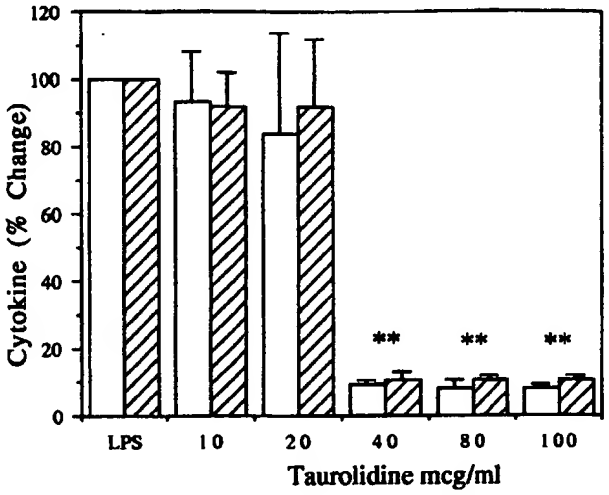


**Figure 7. Effect of preincubation of PBMC with taurolidine on LPS induced TNF (□) and IL-1 (▨) synthesis.**

PBMC were preincubated with increasing concentrations of taurolidine for 30 min at 37°C as shown on the horizontal axis and then subsequently stimulated with LPS (10 ng/mL). After 24 h of incubation, total TNF and IL-1 synthesis were measured. The data represent the mean percent change (±SEM) in cytokine synthesis in PBMC from three donors. $^{**}P < 0.01$.

synthesis of IL-1 and TNF. These data are also consistent with the hypothesis that the ability of taurolidine to reduce LPS fever in rabbits is due, in part, to its suppression of synthesis of the pyrogenic cytokines IL-1 and TNF; however, the antiinfectious actions of taurolidine could be due to both its antitoxicity as well as its ability to suppress these cytokines.

Previous studies were based on the concept that taurolidine "neutralizes" LPS through its ability to cross-link amino groups in various toxins through the

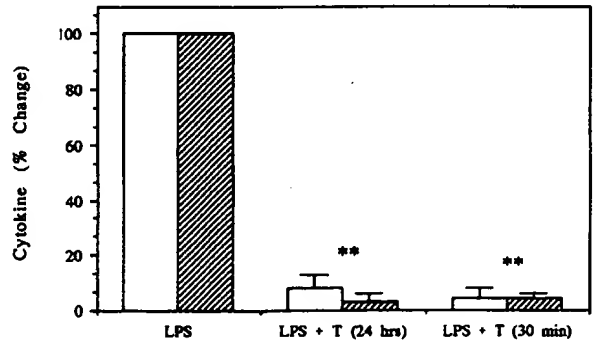


**Figure 8. Effect of short-term exposure to taurolidine on LPS-induced IL-1 (▨) and TNF (□) synthesis.**

PBMC were incubated with taurolidine (80 μg/mL in RPMI) for 30 min at 37°C, centrifuged, and the supernatant was removed. The cells were then resuspended in fresh RPMI and challenged with LPS (1 ng/mL). After 24 h at 37°C, the cells were lysed and assayed for IL-1β and TNF. The data represent the mean percent change (±SEM) in cytokine synthesis in PBMC from three donors. $^{**}P < 0.01$.

release of active methylol groups.[16-19] Indeed, there is evidence that such cross-linking of proteins is a property of taurolidine. However, the ability of LPS to induce IL-1 and TNF is not due to primary amino groups in LPS but rather the negative charges of the lipid. A moiety provided by the fatty acid side chains.[20] Nevertheless, we designed our initial experiments to allow prior incubation of LPS with taurolidine in order for cross-linking to occur. Following this pretreatment, LPS was then added to suspensions of human PBMC. A clear reduction in TNF and IL-1 production from PBMC was observed. The data showed that the ability of taurolidine to reduce the synthesis of IL-1 and TNF was unrelated to the molar ratios of taurolidine to LPS but rather to the absolute amount of taurolidine added to the PBMC. Indeed, suppression of IL-1 and TNF synthesis by taurolidine was most effective when the compound was incubated with the PBMC before being stimulated with LPS. Furthermore, following this preexposure (30 min), taurolidine could be washed from the cells and the subsequent 24 h of IL-1 and TNF synthesis was still suppressed to the same degree as that when taurolidine was present for the entire 24 h.

We were able to show that the effective concentrations of taurolidine in these mononuclear cell cultures which reduced IL-1 and TNF synthesis were not toxic as measured by the sensitive release of LDH. Concentrations of 100 μg/mL of taurolidine reduced synthesis of these cytokines by 80 to 100% without significant increases in the release of LDH. These data are consistent with clinical studies as well as animal toxicity data which have shown that taurolidine is well-tolerated and manifests no toxic side effects.[14] Although we cannot interpret the data derived from in vitro concentrations of taurolidine greater than 100 μg/mL, it is interesting to note that in some clinical trials, local application of taurolidine in a gel-base to open wounds may deliver concentrations greater than 100 μg/mL.

Although it can be argued that the ability of taurolidine to reduce the synthesis of IL-1 and TNF induced by LPS or TSST-1 is mediated by an antitoxic property by which cross-linking of toxic moieties reduces the potency of the toxins, the reduction by taurolidine of TNF synthesis induced by heat-killed *S. epidermidis* strongly suggests that the compound is exhibiting its effect on the cytokine-producing cell rather than the cytokine-inducing agent. *S. epidermidis* does not produce toxins and in these experiments we used heat-killed organisms, thus eliminating a role for toxin-induced cytokines. The mechanism by which heat-killed *S. epidermidis* induces cytokine gene expression and synthesis is not entirely known but likely involves its particulate nature. The organisms are phagocytized and the amount of cytokine induced by heat-killed organisms is, in part, related to phagocytosis. Other phagocytic particles such as zymosan, also induce cytokines. Therefore, the ability of taurolidine to reduce cytokine production induced by heat-killed *S. epidermidis* appears to be independent of its antitoxic property.

One possible mechanism of taurolidine's ability to reduce cytokine synthesis is suppression of translation. Agents which increase cAMP content of cells will suppress cytokine translation.[21-23] It is possible that taurolidine's effect on PBMC is related to changes in cyclic nucleotides. In this study, we observed an increase in TNF and IL-1 production induced by TSST-1 at low (10 to 20 μg/mL) concentrations of taurolidine before a suppressive effect at 80 and 100 μg/mL; this bimodal effect is often observed with agents that increase cAMP in cells. Another possible effect is a temporary, reversible change in cellular pH. The concept that taurolidine suppresses translation rather than transcription is supported by the observation that taurolidine added at the same time as LPS to PBMC suppressed cytokine synthesis. In general, a putative effect on reduction in cytokine gene expression would require prior incubation of taurolidine with PBMC before the stimulation with LPS. Nevertheless, suppression of gene expression is also a possible mechanism because we observed a dramatic effect on cytokine synthesis when PBMC were incubated in the presence of taurolidine before being stimulated with LPS. It is possible that both mechanisms are involved as is the case in histamine-induced suppression of TNF.[22] Further studies can be designed to study the effect of taurolidine on cytokine synthesis. However, the present data provide an important and novel mechanism by which taurolidine may be beneficial in the treatment of cytokine-mediated diseases such as bacterial shock and certain inflammatory disorders.

## MATERIALS AND METHODS

### *Materials*

Ficoll was purchased from Sigma (St. Louis, MO) and prepared with water that had been subjected to ultrafiltration using polysulfon hollow fiber filters.[24] Hypaque (Winthrop Brean, New York, NY) was diluted in ultrafiltered water. RPMI (Gibco, Grand Island, NY) was dissolved in ultrafiltered water and $Na_2HCO_3$, 100 μg/mL streptomycin, 100 U penicillin/mL and 0.01 M HEPES (*N* -2-hydroxyethylpiperazine-*N* '-2-ethanesulfonic acid) buffer (all from Gibco) were added. This RPMI was then subjected to ultrafiltration and stored in pyrogen-free plastic flasks at 4°C. LPS (B:055) was purchased from Sigma; purified TSST-1 was kindly supplied by Dr. Bergdoll, University of Wisconsin, Madison, WI. Previous studies from this laboratory have shown TSST-1 to be a potent stimulator of TNF and IL-1 synthesis.[25] *S. epidermidis* was obtained as a clinical isolate, grown for 24 h in brain heart infusion broth (Difco, Detroit, MI), centrifuged, washed three times in pyrogen-free saline and boiled

for 30 min. When the bacterial suspension had cooled, it was washed an additional three times and then adjusted to optical density of 1.0 at 450 nm. Dilutions of the suspension were made and adjusted to approximately $5 \times 10^7$ bacterial particles per milliliter. When added to equal volumes of PBMC, the final bacteria:leukocyte ratio was 10:1. Human AB serum was prepared from a single donor under pyrogen-free conditions, heated at 56°C for 30 min, filtered through 0.22 μm, and stored at −20°C. Bovine serum albumin (radioimmunoassay grade) was purchased from Sigma. Goat anti-rabbit IgG was purchased from Rockland Laboratories, Rockland, PA. Polyethylene glycol (MW, 6 to 8,000) was obtained from Fisher, Medford, MA. Radiochemicals were purchased from New England Nuclear, Boston, MA.

Taurolidine was provided by the manufacturer (Geistlich Pharm) as a 2% sterile solution in kollidon (Taurolin, Geistlich Pharm). The vehicle kollidon (5% polyvinyl povidone, I..G. Farben-BASF) was also provided. The vehicle was diluted to the same extent (vol/vol) in RPMI as the taurolidine solution.

## *PBMC*

Venous blood from healthy, overnight fasting donors was obtained in heparinized (10 U heparin/mL) (Abbott, North Chicago, IL) syringes. This was mixed with 2 parts of 0.9% pryogen-free saline (Baxter, Deerfield, IL) and 30 mL was added to each 50-mL polypropylene tube (Falcon, Oxnard, CA). The diluted blood was underlayered using a spinal needle with 10 mL of Ficoll-Hypaque mixture and centrifuged at 450 × *g* at room temperature for 40 min. The interphase containing the mononuclear cell fraction was removed, washed in pyrogen-free saline twice, and resuspended in RPMI. The cell concentration was determined on a hemocytometer and adjusted to $5 \times 10^6$ cells/mL. AB serum to make a final concentration of 2% (vol/vol) was added and the cells placed at 4°C until used.

In some experiments, microbial stimulants were preincubated with taurolidine or vehicle in 5 mL polypropylene tubes (Falcon) for 60 min at 37°C and then equal volumes (0.5 mL) were added to equal volumes of PBMC (0.5 mL) in 1 mL flat bottom 24-well culture plates (Falcon). The final concentration of PBMC was $2.5 \times 10^6$/mL, 1% AB serum. In the Results section, all concentrations of microbial stimulants or taurolidine are expressed as final concentrations during the overnight PBMC culture. After 24 h, 37°C in 5% $CO_2$ humidified atmosphere, the plates were frozen at -70°C. Before RIA for IL-1β and TNF-α, the plates were thawed and subjected to two more freeze and thaw cycles in order to obtain total cell synthesis (supernate plus cell associated) of each cytokine. Previous studies have described optimization of this method for total cytokine synthesis.[26] Lactic acid dehydrogenase (LDH) assay was performed as previously reported.[27]

## *RIA*

The RIA results for human IL-1β[26] and TNF-α[28] have been published previously. Briefly, dilutions of the PBMC cultures were made in RIA buffer (0.25% BSA, 0.05% sodium azide, 0.01 M phosphate buffered saline) in order to obtain inhibition between 40 and 80% on the standard curve. After 24 h at room temperature with the first antibody (anti–IL-1β or anti–TNF-γ), samples were pulsed with the respective $^{125}$I-labeled cytokine. After an additional 24 h, samples were precipitated with 2% goat–anti-rabbit IgG, 6% polyethylene glycol and 1% normal rabbit serum, centrifuged at 1,000 × *g* at 4°C, and decanted. The precipitates were counted in a gamma counter.

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Dr. Wolff is a member of the Board of Scientific Advisors of Carter-Wallace, Inc.

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